Colette PAUPARDIN*†, Claude LEDDET** & Roger GAUTHERET***:

Histo-chemical investigations on fennel. Physiological connection between terpen's nature and histological structure of secretory apparatus (2)****

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ウイキョウの組織化学的研究. テルペン類と分泌器官の組織学的・生理学的関連 (2)

B. Questions and problems raised by the comparison between chemicals and histological results. Chemical analysis revealed important differences according to organs. These differences are quantitative and even qualitative.

Differences were parallelelly observed about secretory canals structure. Comparison of the two classes of results suggests that essence composition is not specific of organs but of the kind of canals. On the other hand, the variations observed along a plant are hardly compatible with the existence of connections between secretory canals of the different organs.

Indeed the compounds of essential oil are easily diffusible and their confinement in a given organ suggests that the secretory system is composed of independent units. Perrot has reported that there is no connection between fruits canals and those of the peduncle. We undertook to verify this assertion and to consider in detail, the discontinuity between root and stem which is very typical.

I. Analytical and morphological discontinuity at collar level. Passage from root to stem is characterized by a very important modification of oil composition; the root has no anethol and contains dillapiol, while, it is the contrary for the stem. The change of composition occurs on a few millimeters.

If there were anastomoses between the secretory structures of the two organs, a diffusion would surely take place and this would not present drastic differences in the composition of oils coming from the stem and the root.

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In order to verify that there are no connections between the secretory systems of the root and stem, we have confronted analytical and histological results, about the limit of the two organs, that is to say the collar. This investigation was realized with sweet fennel and samples were taken on a 30 cm plant. One at the collar itself. The second just above the stem foot and the third at the first ramification of the stem. The three samples were deprived of chlorophyl, because there was hypogeus.

Collar's essential oil contains 88% of dillapiol. This concentration is the same as in the root. The essential oil extracted from the stem foot contained only 58% of dillapiol and 10% of anethol. Finally, at the first branching of the stem, the essential oil contains no more than 20% of dillapiol and 39% of t-anethol.

This abrupt variation takes place on a length lower than one mm. Its interpretation requires obviously very careful histological observations. These observations were realized on a lot of transversal sections distant of 60 μm. It was seen that the stem has an epidermis and a pith, whereas the root has a periderm and no pith. The transition from epidermis to periderm over-spreads a length of about 180 μm; and the pith extends 900 μm after the end of the epidermis. This value is the total length of the collar.

Secretory canals located at the foot of the stem are similar to the canals of the upper part. However, they are not numerous and the collar itself has only a few canals. Those of the cortex has the same structure than in the root. In the upper part of the root, the canals offered a very peculiar aspect. They are twisted, and instead of being parallel to the organ axis, their lower extremity is disposed orthogonally with respect to this axis.

Finally, superposition of serial sections allowed to follow canals through the collar and its contiguous regions. This led to notice that the secretory canals are very short. The length of most of them does not exceed 120 μm and only a few reach 400 μm. Moreover, these canals are independent and not anastomosed. Therefore, variations of essential oil composition are understandable, because they are the result of progressive substitution of "stem" canals by "root" canals.

II. Canals discontinuity between fruits and peduncles. We tried to verify Perrot's work who reported that there is no junction between the secretory system of fruit and this of its peduncle. This verification has been achieved thanks to serial sections involving the bottom of the fruit and the top of the peduncle.

The distance between each section was about 60 μm. Fig. 2 which summarizes the observations, shows clearly that along some hundred μm, the fruit peduncle has no secretory canals and that there is no connection between canals of the fruit and those of its peduncle, which are furthermore not numerous and very narrow.

III. Discontinuity of secretory at the level of the stem branching. The kind of fennel's branching suggested that new secretory canals must differentiate at each ramification and we tried to verify this thought.

Branching of the stem is sympodial. In other words, the ramification occurs

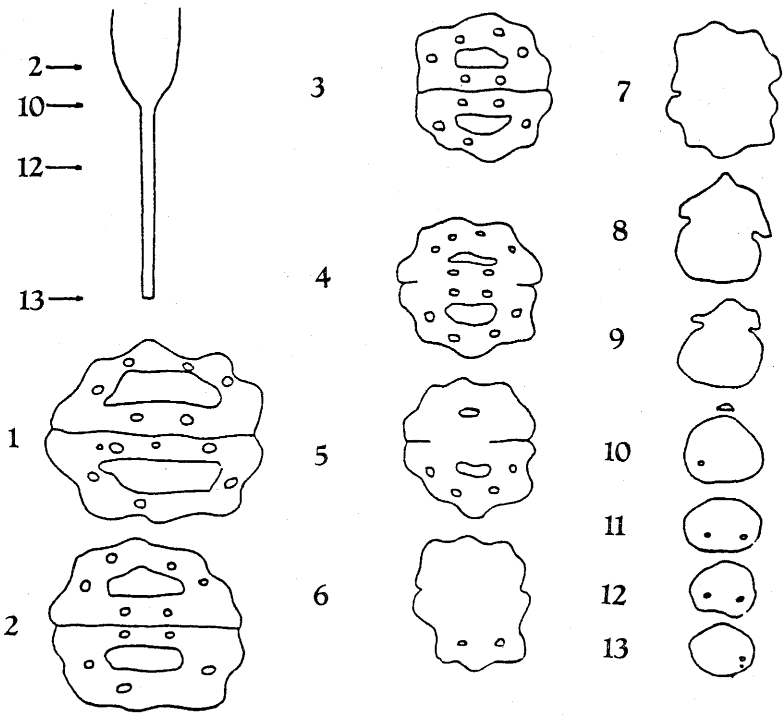

Fig. 2. Transverse sections of a fruit and its peduncle. It is clear that there is no connection between fruit and peduncle canals. Bottom of the fruit has no canals at all and only two narrow canals can be seen in the peduncle.

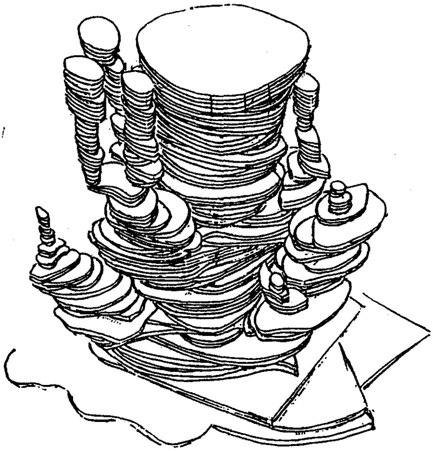

Fig. 3. Space representation of fennel stem branching. This pattern was built by superposition of numerous transverse sections of a stem tip. The sympodial branching can be distinguished.

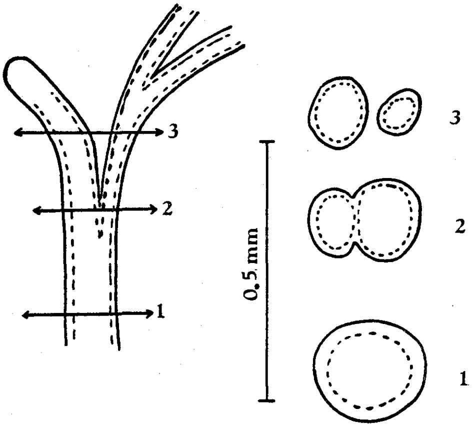

Fig. 4. Diagram showing newly-formed secretory canals at branching level. 1) Under the branching, the stem cortex contains secretory canals disposed approximately on a ring. 2) When branching is prepared, new canals differentiate. 3) Above the branching point, the new canals share out among the two stems.

thanks to an unequal partition of the apex. Thus, are formed two meristems. The smaller one develops into a thin stem, which does not durably elongate. On the other hand, the main meristem grows vigorously and it is transformed into a stem which can ramify continuously in the same sympodial manner. (Fig. 3)

Under the ramification, the stem cortex contains numerous secretory canals approximately arranged on a circumference. Observing serial sections of the branching region, one can notice the appearance of newly formed canals in the internal face of the two branches and these canals will be distributed between these branches (Fig. 4). Numerous observations revealed that the newly formed canals were quite independent. At the level of branching and just under the cleaving point of the meristematic region, the canals are very short. Their average length is about 120 μm. Some of them measure less than 50 μm and only a few canals exceed 400 μm.

C. In vitro experiments.

1) The confrontation between analytical and histological data has thrown into relief the fact

that secretory canals are specific chemically and morphologically. It seemed interesting to wonder whether in *in vitro* culture, the two kinds of specificities could be dissociated or not.

This experiment was realized on sweet fennel. We used a strain of callus produced by a stem segment and this was transferred many times. The culture medium consisted of mineral salts of Murashige and Skoog's solution supplemented with glucose (3%), naphtalene acetic acid (10^{-7} g ml^{-1}), benzyladenine (10^{-6} g ml^{-1}), a mixture of vitamins: inositol (5 mg l^{-1}), nicotinic acid (0.25 mg l^{-1}), thiamine (0.5 mg l^{-1}), pantothenic acid (0.5 mg l^{-1}), pyridoxine (0.25 mg l^{-1}), folic acid (0.1 mg l^{-1}), biotine (0.25 mg l^{-1}) and glycine (1 mg l^{-1}). This nutrient was solidified with agar (6 g l^{-1}). The cultures were kept at 22°C and exposed to light (2,500 lux). Explants developed quickly and gave colonies made of callus tissue, capable producing buds which transformed into stems.

Buds and callus were analysed separately. The callus was very poor in essential oil and trans-anethol content was particularly low (Tab. 5). On the contrary, the newlyformed buds synthesized more essential oil, which was specially rich in limonene and trans-anethol (Tab. 5). Concentrations of the other components were very low in both callus and buds.

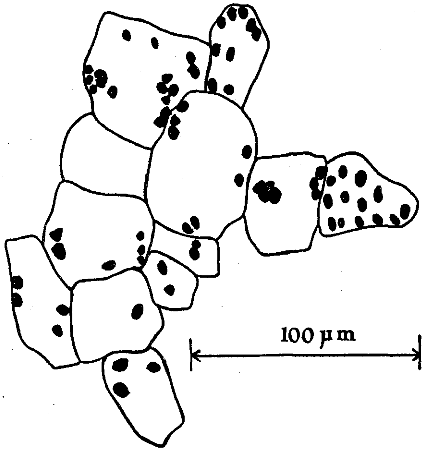

Fig. 5. Cells of a callus obtained by *in vitro* culture of Sweet Fennel stem tissue. There are no secretory canals but cells of the callus contained small drops of essential oil, which are black-stained by O_sO_4 used as fixative.

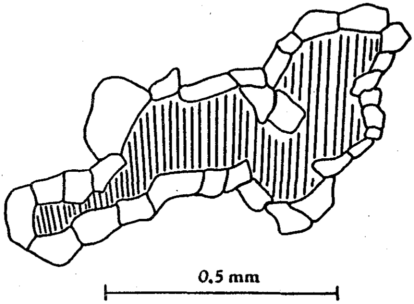

Fig. 6. Abnormal secretory canal differentiated at the bottom of a stem of Sweet Fennel growing *in vitro*. This canal was pre-existing in the cultured stem, but its shape has been deeply modified by the *in vitro* culture.

Tab. 5. Composition of essential oils extracted from callus and buds grown *in vitro*. Amounts expressed in μg/g of fr. wt.

	limonene	estragol	cis-anethol	trans-anethol	anisaldehyde	phenol	dillapiol
Buds	252	9	3	216	1	11	14
Callus	9	0	5	4	0	12	3

Histological observations revealed no presence of secretory canals in the callus itself. But many cells inclosed small granulations capable of reducing osmic acid and which, obviously, consist of essential oil (Fig. 5).

On the other hand the newly formed stem of the callus has quite normal secretory canals and concurrently, they synthesize t-anethol. However, at the bottom of the stem, abnormal canals can be seen, canals which were enlarged, twisted and inserted between stem canals and root secretory pockets (Fig. 6). It seems that these abnormal secretory canals correspond to the special essential oil composition mentioned in Tab. 5.

Finally, these experiments demonstrate that morphological and chemical specificities of secretory cells can be modified by *in vitro* culture, but not completely dissociated. (To be continued)

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2種類のウイキョウ bitter fennel (*Foeniculum vulgare* Miller var. *vulgare*) と sweet fennel (*F. vulgare* Miller var. *dulce* (Miller) Thellung) を材料として，根，茎，葉，花柄，果実各器官における精油含量・組成の分析，及び組織学的観察を行った。精油組成と組織学的観察の違いなどから，分泌管は独立した単位からなっており，器官間で接続していないと考えられた。そこで，根と茎の間，果実と花柄の間及び茎の分枝部分において分泌管が接続しているか否かについて sweet fennel で検討した。連続切片を用いた組織学的観察から，いずれの境界領域でも分泌管は短くて独立していることが判明した。更に，分泌管が分泌する精油成分の特性とその形態学的特性の関係を in vitro 培養を用いて検討した。茎から誘導したカルスは，精油含量は非常に低く分泌管は観察されなかったが，細胞内には精油を含有する小粒が存在した。一方，カルスから再分化した不定芽と茎には正常な分泌管が存在したが，茎の基部では異常な分泌管も観察された。